



IMPROVED TEMPERATURE SENSITIVE MICELLES

5 Field of the Invention

The invention relates to a temperature sensitive micelle system. More particularly, it relates to a temperature sensitive micelle system for improving the encapsulation and delivery of hydrophobic drugs.



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Background of the Invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date:

- 15 (i) part of common general knowledge; or
(ii) known to be relevant to an attempt to solve any problem with which this specification is concerned.

Targeting a drug to selected tissues and cells, especially for cancer therapy, is one of the greatest challenges in controlled release since most anticancer drugs have poor water solubility. One approach that has been adopted is drug conjugation to polymeric carriers, however the targeting abilities of the drug-polymer conjugates may be substantially lowered in living bodies because of drug-carrier conjugate scavenging by the reticuloendothelial system. Other approaches such as the use of prodrugs, micelles, lipoproteins and viral carriers to deliver drugs/genes to a specific location in situ have been investigated. However, obtaining regulatory approval for prodrugs is complicated and difficult, while viral carriers are likely to elicit an immune response. Synthetic lipoproteins targeted against receptor sites on tumors may also be recognised by receptors on healthy cells, thus making their effects non-specific. Polymeric micelles offer a better alternative to these methods.

30 Some research has been conducted into polymeric micelles in order to attempt to achieve longer circulation in the blood stream and to inhibit the above mentioned scavenging by the reticuloendothelial system.

AB-type block copolymers having both hydrophilic and hydrophobic segments are known to form micellar structures in aqueous media due to their amphiphilic character. Highly hydrated outer shells of the polymeric micelles can inhibit intermicellar aggregation of their hydrophobic inner cores. As a result, the polymeric micelles are able

to maintain satisfactory aqueous stability irrespective of the high content of hydrophobic drug incorporated within the micelle inner core.

Poly(N-isopropylacryamide) (PNIPAAm) is a known water-soluble polymer, which shows reversible hydration-dehydration changes in response to relatively small temperature changes of the relevant solution. A temperature showing the hydration-dehydration change is called the lower critical solution temperature ("LCST"). PNIPAAm is water-soluble and hydrophilic and shows an extended chain confirmation below its LCST and it undergoes a phase transition to an insoluble and hydrophobic aggregate above the LCST. This phase transition occurs with narrow temperature changes through the LCST and is reversible. Hydrophobic chains of collapsed PNIPAAm above the LCST can actively interact with bio components, such as cells and proteins, or other hydrophobic components, whereas below the LCST, hydrated flexible chains do not interact with them.

AB - type block copolymers of PNIPAAm and a hydrophobic segment exhibit thermo-responsive water solubility and can form micellar structures composed of hydrophilic micro domains of soluble PNIPAAm segments together with hydrophobic aggregated micro domains of incorporated hydrophobic segments in aqueous solution below the LCST. The hydrophobic inner core of the micelle may contain a drug (e.g. a hydrophobic drug), while the PNIPAAm outer shell plays a role of stabilization and temperature response. The hydrophilicity of the outer shell inhibits the inner core from reacting with bio components. This hydrophilicity can be switched to hydrophobicity at a designated site by temperature increasing through the LCST.

Micelles are small-sized and can solubilize hydrophobic drugs, genes or proteins in their inner cores through hydrophobic interaction, electrostatic interaction and hydrogen bonding etc., while exposing their hydrophilic shells to the external environment. This effectively protects the enclosed bioactive compounds against degradation and enables them to exhibit prolonged activity in the systemic circulation of the body. With micelles, targeting can be achieved, both passively and actively, through an enhanced permeation and retention effect (EPR) and the incorporation of recognition signals to the surface of the vesicles, or inducing a polymer sensitive to variation in temperature or pH. Because of their distinct advantages mentioned above, polymeric micelles are an ideal carrier for hydrophobic drugs or genes.

It is known that PNIPAAm in aqueous solution exhibits a reversible thermo-responsive phase transition at about 32°C, and therefore this is the LCST of this polymer. As noted above, the polymer is water soluble and hydrophilic below its LCST and undergoes a phase transition to an insoluble and hydrophobic aggregate above the LCST. The

thermo-responsive properties of PNIPAAm therefore allow its utilisation as an on-off switch for drug release.

Chung, J.E et al have reported the fabrication of thermo-responsive polymeric micelles made from block copolymers of PNIPPA_m with either poly(butyl methacrylate) or polystyrene. The PNIPPA_m-poly(butyl methacrylate) micelles were able to release an anticancer drug only when heated above the LCST of the polymer. However, such micelle systems are not degradable and do not contain functional groups for ligand conjugation for active targeting.

Summary of the Invention

The present inventors have developed a micelle system formed of block copolymers containing cholesteryl and temperature-sensitive moieties as well as a more hydrophilic segment compared with the temperature-sensitive moiety. The more hydrophilic segment may contain functional groups for ligand conjugations. It has been found that the length of the hydrophilic segment can affect the LCST of the copolymers in solution and, therefore, by controlling the length of the hydrophilic segment, one can adjust the LCST of the copolymers. The above micelle systems can improve water solubility of hydrophobic drugs via physical encapsulation. They also exhibit good stability in aqueous media, adjustable LCST and high drug loading capacity.

The micelle system of the present invention is capable of undergoing a phase transition at its lower critical solution temperature (LCST). Preferably, the LCST values are in the range of from 37°C to 45°C, which are slightly higher than body temperature. The LCST value of micelles should be higher than body temperature. Otherwise, the micelles would aggregate (and drug molecules would be released) after injection into the body because the shell of micelles is water-insoluble at temperatures higher than the LCST. Thus, the shell of the micelles becomes hydrophobic and drug molecules are released out because of the de-association of micelles when the local temperature is increased slightly (higher than body temperature).

The above LCST values are higher than the LCST values of the copolymers used in some earlier polymeric systems. These higher LCST temperatures result in the micelles having greater stability in the blood system of a patient. This greater stability improves the delayed release capabilities of the micelle system which, in turn, improves the desired targeting of the relevant drug.

Tailoring of the length of the more hydrophilic segment can be used to adjust the LCST. The higher the molecular weight of cholesteryl-terminated P(NIPAAm-co-DMAAm), the longer the P(NIPAAm-co-DMAAm) segment is. Usually, the LCST is adjusted by varying the ratio of NIPAAm to DMAAm in the copolymeric molecule.

According to a first aspect of this invention, there is provided a polymeric micelle system, for encapsulating a hydrophobic substance, wherein each polymeric micelle is formed of block copolymers each having a temperature-sensitive block and a cholesteryl moiety, wherein the cholesteryl moieties form a hydrophobic core of the micelle and the temperature-sensitive blocks form a hydrophilic shell of the micelle.

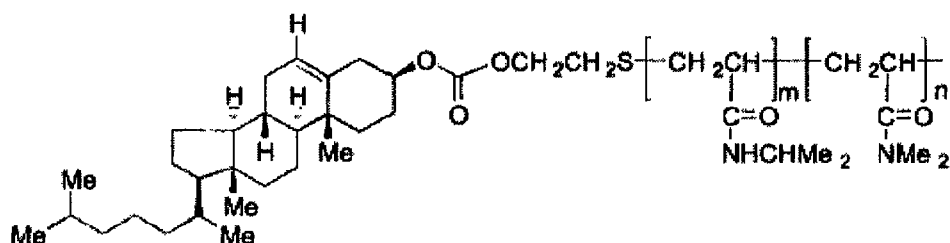
Preferably, the temperature-sensitive block consists of a temperature-sensitive segment and a more hydrophilic segment. The temperature-sensitive segment is preferably poly(N-isopropylacrylamide) ("PNIPAAm") or one of its derivatives such as poly(N,N-diethylacrylamide). The more hydrophilic segment preferably comprises one or more functional groups for ligand conjugations. One of the reasons why cholesterol is chosen as the hydrophobic segment is because of its potential interactions with cholesterol receptors on a cell surface and its ability to drive self-assembly in aqueous solutions. The more hydrophilic segment may be selected from the group consisting of poly(N, N-dimethylacrylamide) ("PDMAAm"), poly(N-(hydroxymethyl)acrylamide) ("PNHMAAm"), poly(acrylic acid), poly(vinylacetic acid), and their derivatives. The derivatives of the abovementioned molecules include those containing amine groups (eg poly((dimethylamino)ethyl methacrylate)), hydroxyl groups or carboxylic acid groups. It is particularly preferred that the more hydrophilic segment is PDMAAm.

In an alternative embodiment, the more hydrophilic segment is PNHMAAm.

In another alternative embodiment, the more hydrophilic segment is poly(acrylic acid) or poly(vinylacetic acid) or poly((dimethylamino)ethyl methacrylate).

The cholesteryl moiety may be cholesterol or a cholesterol derivative. In this embodiment, the cholesteryl moiety and the temperature-sensitive block are linked by a carbonate bond. Such a carbonate bond is potentially hydrolytically labile which helps make these copolymers biodegradable in aqueous media.

In one preferred embodiment of the invention, the block copolymer is cholesteryl-terminated poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide), having the formula:



wherein the ratio of m to n is in the range of 100:1 to 1:100.

In one preferred embodiment, the ratio of m:n is about 7:1.

Preferably, the value of m will be between 1 and 1000 and the value of n will be

- 5 between 1 and 1000. In one particular embodiment, the value of m is 31 and the value of n is 4.

Typically the micelles are capable of undergoing a phase transition at a lower critical solution temperature ("LCST") in the range of from about 32°C to about 50°C, and preferably from 37°C to 45°C.

- 10 The above micelle system generally has a critical micelle concentration (CMC) in the range of from 4 mg/L to 200 mg/L. This low CMC indicates that the system is highly stable in aqueous media.

In a further embodiment of this invention, the polymeric micelle system further comprises a hydrophobic substance encapsulated with the polymeric micelles.

- 15 The hydrophobic substance is typically a drug selected from the group consisting of anti-cancer drugs, antiphlogistic anodynes, immuno-suppressants, hepatism remedies, hormone compositions, chemo-therapeutics, metabolic pharmaceuticals, digestive disease remedies, respiratory disease remedies, anti-allergic pharmaceuticals, central nervous system disease remedies, peripheral disease remedies, circulatory disease
20 remedies and anti-fungal remedies.

In one preferred embodiment, the drug is an anti-cancer drug selected from the group consisting of paclitaxel, doxorubicin, teniposide, etoposide, daunomycin, methotrexate, mitomycin C, indomethacin, ibuprofen, cyclosporin, biphenyl dimethyl dicarboxylate, triamcinolone acetonide, betamethasone, amphotericin B, ketoconazole, itraconazole,
25 daunorubicin, griseofluvin, foroprimidine, lidocaine, epirubicin, vincristine, vinblastine, ellipticine, camptothecin, docetaxol, cisplatin, prednisone, and methyl-prednisone.

Particularly preferred anti-cancer drugs include cyclosporin A, paclitaxel and doxorubicin.

The micelle system is capable of improving water-solubility of hydrophobic drugs, and for temporally and spatially targeted delivery of hydrophobic drugs. The temporally and spatially targeted delivery is achieved through temperature-sensitivity and interactions between the conjugated ligands and target cells. The interactions between the conjugated ligands and target cells would promote delivery of the micelle to the specific tissues. Heating the tissues to a temperature of above the LCST would trigger drug release from the micelle.

In another aspect of this invention, there is provided a method of preparing the block copolymers described above, in which the more hydrophilic segment is PDMMAm, said method comprising the following steps:

- (a) synthesising hydroxyl-terminated poly(N-isopropylacrylamide-co-N, N-dimethylacrylimide) precursor polymers; and
- (b) reacting the precursor polymers with cholesteryl chloroformate.

It is contemplated that other cholesteryl compounds (as alternatives to cholesteryl chloroformate) could also be used.

The reaction of step (b) is typically an esterification reaction which is catalysed by a suitable catalyst. The catalyst may be triethylamine. Preferably, the esterification reaction is carried out at room temperature.

The above reaction is preferably effected by dissolving the cholesteryl chloroformate, the precursor polymers and the catalyst in a solvent (such as CaH₂-dried dichloromethane) to form a reaction mixture. The reaction mixture is preferably stirred for about 24 hours at room temperature.

The above method may further include a precipitation step to precipitate the block copolymers from the reaction mixture. The precipitation may be effected by adding diethyl ether to the reaction mixture. The above method may further include a purification step wherein the precipitated products are purified by ultra-filtration using a filtration membrane. This filtration membrane typically has a molecular weight cut-off of about 2000.

In a preferred embodiment of this method, about 1 gram of hydroxyl-terminated P(NIPAAm-co-DMAAm), about 0.5 grams of cholesteryl chloroformate and about 0.1 gram of triethylamine are dissolved in 30 mls CaH₂ – dried dichloromethane.

The precursor polymers used in step (a) of the above method may be synthesised by radical copolymerisation of N-isopropylacrylamide and N,N-dimethylacrylamide

monomers, in the presence of an initiator and a chain transfer agent. A preferred initiator is benzoyl peroxide. A preferred chain transfer agent is 2-hydroxyethanethiol.

In another aspect of this invention, there is provided a method of preparing the block copolymers described above, in which the more hydrophilic segment is PNHMAAm, said method comprising the following steps:

- (a) synthesising poly(N-isopropylacrylamide-co-N-(hydroxymethyl)acrylamide precursor polymers; and
- (b) reacting the precursor polymers with cholesteryl chloroformate.

The reaction of step (b) is generally an esterification reaction as described above. This reaction may be catalysed using triethylamine.

The precursor polymers and the cholesteryl chloroformate (and any catalyst) are preferably dissolved in a solvent, such as dichloromethane or tetrahydrofuran.

For the synthesis of the cholesteryl-terminated polymer, the reaction is typically conducted at room temperature for about 24 hours.

For the synthesis of the cholesteryl-grafted polymer, the reaction mixture is typically refluxed at about 70 °C for about 6 hours.

Before step (a) of this method, the precursor polymers may be synthesised by co-polymerising NIPAAm and NHMAAm in the presence of an initiator (typically benzoyl peroxide).

A membrane dialysis process may be used for micelle fabrication and drug encapsulation. Moreover, one can use a solvent evaporation method to fabricate the micelles and perform drug encapsulation as well. In addition, one can change the fabrication conditions such as solvent nature, polymer concentration and dialysis membrane to achieve the same purpose.

Accordingly, in another aspect of this invention, there is provided a method of preparing the polymeric micelles of the system described above, comprising the following steps:

- (a) dissolving the block copolymers in dimethylformamide ("DMF") to form polymer solutions; and
- (b) dialyzing the polymer solutions against de-ionized water.

The dialysis step is generally effected by membrane dialysis. A dialysis membrane with various molecular weight cut-offs can be used. Particularly good results have been achieved using membranes having a molecular weight cut-off of about 2000. The dialysis membrane is typically a dialysis bag.

In this method, the initial concentration of the block copolymers in DMF is preferably about 0.35% by weight. The micelles formed by this method are typically cuboid-like in shape and have a size in the range of about 45-80 nm for the low Mw polymer and

about 70-110 nm for the high Mw polymer. The molecular weight of the polymer affects micelle size and the preferred polymer concentration may vary for polymers with different molecular weights.

Where the initial concentration of the block copolymers in DMF is about 0.1% by weight, the micelles formed tend to be substantially spherical having a size in the range of about 30-50 nm for the low Mw polymer.

Where the initial concentration of the block copolymers in DMF is about 1.2% by weight, the majority of the micelles formed tend to be substantially star-shaped and have a size in the range of about 65-245 nm for the low Mw polymer.

By decreasing the dialysis temperature, micelles of larger size can be formed and the encapsulation efficiency (see below) be increased.

In a further aspect of this invention, there is provided a method for encapsulating a hydrophobic substance into the polymeric micelles, prepared according to the micelle preparation method described above, comprising the following steps:

- (a) preparing a polymer solution of the block copolymers in DMF;
- (b) adding a quantity of the hydrophobic substance to the polymer solution to form a loaded polymer solution; and
- (c) dialyzing the loaded polymer solution against de-ionised water.

Accordingly, drug encapsulation mostly occurs simultaneously with the formation of micelles. Drug encapsulation could, however, be implemented after the formation of micelles, but this will not generally be practical because drug encapsulation efficiency would be very low. The fabrication conditions, such as solvent nature, polymer concentration and dialysis membrane can be varied to optimise drug encapsulation within the micelles.

The dialysis step is preferably effected by membrane dialysis using a dialysis membrane. Again, one can use a dialysis membrane with various molecular weight cut-offs. Good drug encapsulation efficiencies can be achieved using a dialysis membrane having a molecular weight cut-off of about 2000. This dialysis membrane is typically a dialysis bag and the loaded polymer solution is placed in the dialysis bag. The dialysis bag may be immersed in about 500 mls of de-ionised water, typically for up to 8 hours, with the water being replaced about every hour. One can change these conditions yet still achieve high drug encapsulation efficiency.

The dialysis step is generally carried out at a temperature in the range of about 5°C to a temperature of just below the LCST. A preferred temperature is about 10°C.

The hydrophobic substance used in the above encapsulation method is preferably a drug selected from the group consisting of anti-cancer drugs, antiphlogistic anodynes,

immuno-suppressants, hepatitis remedies, hormone compositions, chemotherapeutics, metabolic pharmaceuticals, digestive disease remedies, respiratory disease remedies, anti-allergic pharmaceuticals, central nervous system disease remedies, peripheral disease remedies, circulatory disease remedies and anti-fungal remedies.

It is preferred that the drug is an anti-cancer drug selected from the group consisting of paclitaxel, doxorubicin, teniposide, etoposide, daunomycin, methotrexate, mitomycin C, indomethacin, ibuprofen, cyclosporin, biphenyl dimethyl dicarboxylate, triamcinolone acetonide, betamethasone, amphotericin B, ketoconazole, itraconazole, daunorubicin, griseofulvin, foroprimidine, lidocaine, epirubicin, vincristine, vinblastine, ellipticine, camptothecin, docetaxol, cisplatin, prednisone, and methyl-prednisone. Particularly preferred anti-cancer drugs include paclitaxel, doxorubicin and cyclosporin A. The encapsulation efficiency of cyclosporin A within the micelle system can be achieved up to about 64%.

In another aspect of this invention, there is provided a method of synthesizing block copolymers having a desired LCST, for use in the micelle systems described above, comprising the following steps:

- (a) determining a copolymer ratio of the temperature-sensitive segment to the more hydrophilic segment, in the block copolymer, which is required so that the block copolymer exhibits an LCST which is about the desired LCST;
- (b) preparing a reaction solution comprising monomers corresponding to the temperature-sensitive segment, monomers corresponding to the more hydrophilic segment and an initiator, wherein the molar ratio of the two types of monomers is substantially equal to the copolymer ratio; and
- (c) heating and mixing the reaction solution.

The reaction solution can also include a chain transfer agent. However, a chain transfer agent will not (for example) be required where radical polymerisation is being performed for synthesising cholesterol-grafted temperature sensitive polymers.

In some cases, the molar ratio of the monomers in the reaction solution will be substantially equal to the copolymer ratio.

The temperature-sensitive segment is preferably PNIPAAm.

As stated above, the more hydrophilic segment may be selected from the group consisting of poly(N, N-dimethylacrylamide) ("PDMAAm"), poly(N-(hydroxymethyl)acrylamide), poly(acrylic acid), poly(vinylacetic acid), and their derivatives. The derivatives of the abovementioned molecules include those containing

amine groups (eg poly((dimethylamino)ethyl methacrylate)), hydroxy group and carboxyl acids.

The more hydrophilic segment preferably comprises one or more functional groups for ligand conjugations. The more hydrophilic segment is preferably PDMAAm.

5 In a still further aspect of this invention, there is provided a pharmaceutical composition comprising a polymeric micelle system as described above, and a pharmaceutically acceptable carrier, wherein said micelle system is loaded with a therapeutically effective amount of a hydrophobic drug. Preferably, the hydrophobic drug is an anti-cancer drug and, accordingly, the composition is one for curing, preventing, treating or alleviating
10 cancer. As previously indicated, the anti-cancer drug is preferably paclitaxel, doxorubicin or cyclosporin A.

According to another aspect of this invention, there is provided a method of treating a patient to cure, prevent, treat or alleviate a disease, said method comprising administering to said patient the pharmaceutical composition described above. In a
15 preferred embodiment of this method, the disease is cancer and the hydrophobic drug is an anti-cancer drug, such as cyclosporin A. In this method, the composition may be administered orally, topically, by injection or by suppository.

Accordingly, the above invention provides for a micelle system capable of drug delivery to a target cell by interactions between ligands present on the more hydrophilic segment
20 and the target cell and release of the drug by changes in the environmental temperature surrounding the micelle. The method preferably also includes the step of heating targeted tissue or a targeted organ (eg diseased tissue or a diseased organ) to a temperature above the LCST so as to cause the hydrophobic drug to be released from the micelles.

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Description of the Drawings

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following figures and examples:

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Figure 1 is a graph showing the ^1H NMR spectrum of the low Mw polymer (i.e. polymer 1) in chloroform-D (CDCl_3)

Figure 2 is a graph showing the ^1H NMR spectrum of the low Mw cholesteryl-terminated
35 block copolymer (i.e. copolymer 3) in chloroform-D (CDCl_3).

Figure 3 shows two TEM images (a and b) of micelles formed by the dialysis of a 0.35% initial copolymer solution in DMF, namely micelles of copolymer 3 (see (a)) and of copolymer 4 (see (b)).

5 Figure 4 shows four TEM images (a, b, c, d) of the micellar nanoparticles at initial polymer solutions of 0.1% (see (a)) and 1.2% (see (b) (c) (d)).

Figure 5 shows two TEM pictures (a and b) of CyA-loaded micelles made from copolymer 4 at dialysis temperatures of 20°C (see (a)) and 10°C (see (b)) (6 mg CyA
10 fed).

Figure 6 is a graph showing 'in vitro' release profiles of CyA-loaded micelles with a CyA loading of 12%, fabricated at dialysis temperature of 10°C.

Figure 7 shows a gel permeation chromatogram of the precursor and cholesteryl-grafted
15 polymers, in respect of Example 2. (The retention time is 17.1 and 16.9 min of the precursor and cholesteryl-grafted polymers, respectively.)

Figure 8 is a graph showing ¹H NMR spectrum of the cholesteryl-grafted polymer.

20 Figure 9 shows two TEM pictures of the blank and CyA-loaded cholesteryl-grafted micelles, dialyzed at 10°C.

Detailed Description of Preferred Embodiments

25 The invention may be carried out in the manner set forth in the following examples. As will appreciated, other modes of carrying out the invention are possible without departing from the scope of the invention described above.

EXAMPLE 1: *Cholesteryl-terminated poly(N-isopropylacrylamide-co-N, N-*
30 *dimethylacrylamide) temperature-sensitive micelles*

1.1 Materials

N-isopropylacrylamide and N, N-dimethylacrylamide (purchased from Aldrich) are purified by crystallization (n-hexane) and distillation, respectively. Benzoyl peroxide
35 ("BPO") (from Merck-Schuchardt) and 2-hydroxyethanethiol (from Sigma) are used as

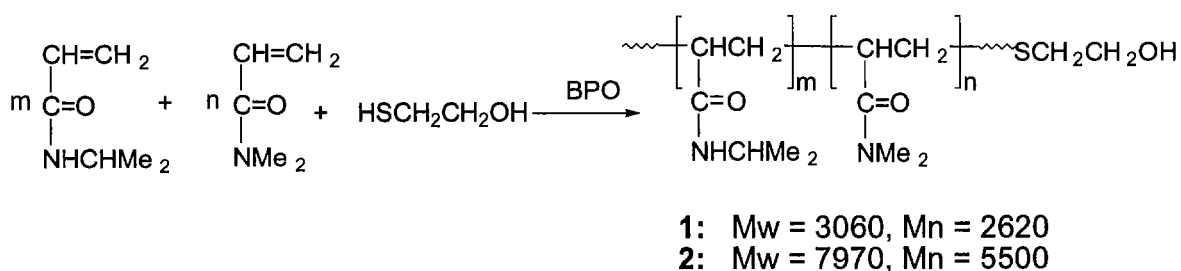
received. Tetrahydrofuran (THF), dichloromethane, diethyl ether and triethylamine are distilled over CaH_2 before use.

1.2 Methods

Synthesis of hydroxyl-terminated poly(*N*-isopropylacrylamide-co-*N*, *N*-dimethylacrylamide)

The two hydroxyl-terminated poly(*N*-isopropylacrylamide-co-*N*, *N*-dimethylacrylamide) precursor polymers are prepared by the radical copolymerization using benzoyl peroxide (BPO) as an initiator and 2-hydroxyethanethiol as a chain transfer agent (Scheme 1). Briefly, *N*-isopropylacrylamide (5.0 g, 44.2 mmol), *N*, *N*-dimethylacrylamide (0.62 g, 6.3 mmol), 2-hydroxyethanethiol (for preparation of a first precursor polymer-40 mg, 0.5 mmol; and for preparation of a second precursor polymer-75 mg, 1.0 mmol); and BPO (21 mg, 0.1 mmol) are dissolved in 20 ml dry THF. The solution is degassed by bubbling with nitrogen for 20 minutes. The reaction mixture is refluxed for 16 h under nitrogen. Upon completion, the product is precipitated out by the addition of diethyl ether. The products are purified by re-precipitation twice from dichloromethane-diethyl ether using a slow liquid-liquid diffusion method. The molecular weights of the polymers are determined by gel permeation chromatography (GPC, Waters. MA USA, polystyrene standards) in THF at an elution rate of 1 mL/min at 25°C.

Scheme 1

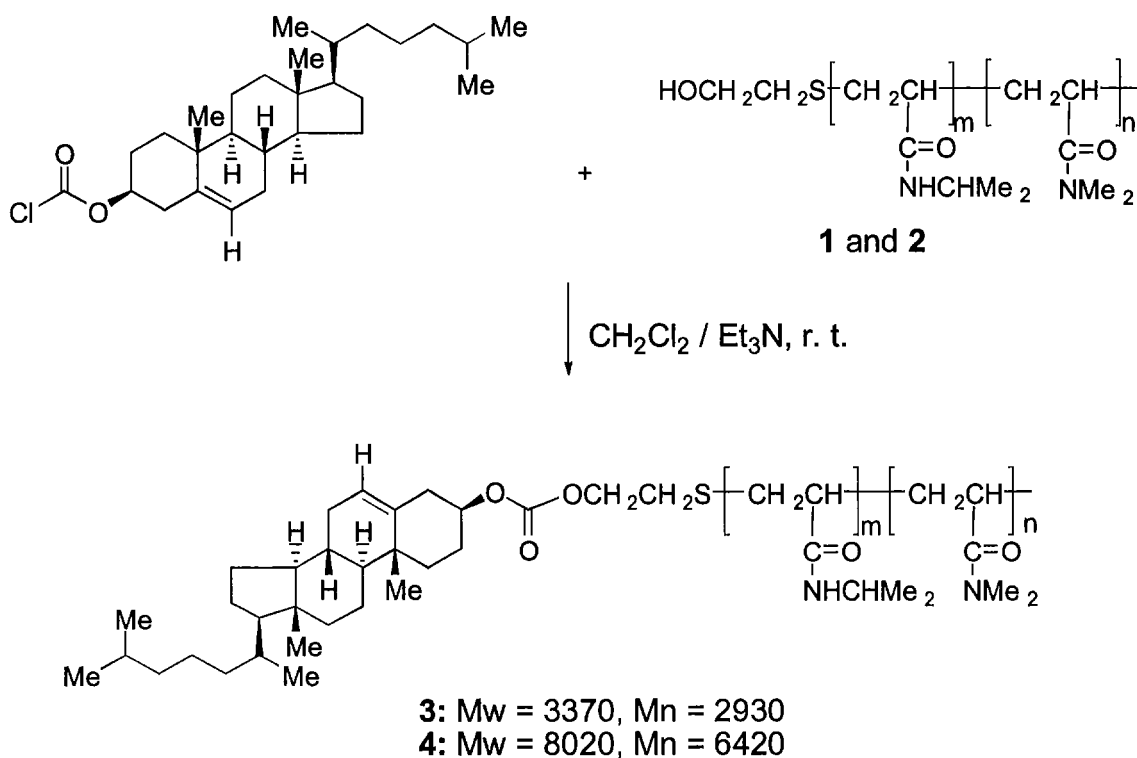


Synthesis of amphiphilic polymers of cholesteryl-terminated poly(*N*-isopropylacrylamide-co-*N*, *N*-dimethylacrylamide)

The cholesteryl-terminated block copolymers are synthesised by the esterification reaction between the precursor polymers and cholesteryl chloroformate using triethylamine as a catalyst at room temperature (Scheme 2). Hydroxyl-terminated P(NIPAAm-co-DMAAm) (1 g), cholesteryl chloroformate (0.5 g) and triethylamine (0.1 g)

are dissolved in 30 mL CaH₂-dried dichloromethane. The reaction mixture is allowed to stir at room temperature for 24 h.

Scheme 2



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Upon completion, diethyl ether is added to the reaction mixture to precipitate the products. The products are purified by ultrafiltration using a filtration membrane with a molecular weight cut-off of 2000 (Sigma D-7884) for 24 h.

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Preparation of micelles

Micelles of cholesteryl-terminated polymers are prepared by a membrane-dialysis method. Briefly, the polymer solutions in dimethylformamide (DMF) are dialyzed against de-ionized water for 24 hours using a dialysis membrane with a molecular weight cut-off of 2000 (Sigma D-7884) at room temperature (typically 20°C to 25° C).

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LCST determination

Optical transmittance of aqueous polymer solutions (5 mg/mL) at various temperatures is measured at 500 nm with a UV-VIS spectrometer (Shimadzu, UV-2501PC, Japan). Sample cells are thermostated with a temperature-controller (Shimadzu, UV-2501PC, Japan). Heating rate is 0.1°C/min. The LCST values of polymer solutions are determined at the temperatures showing an optical transmittance of 50%.

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Determination of critical micelle concentration (CMC)

Fluorescence spectra are recorded on a LS50B Luminescence Spectrometer (Perkin Elmer, USA) at 20°C. The cell holder temperature is controlled with a thermostated circulating bath. Pyrene is used as a hydrophobic fluorescent probe. Aliquots of pyrene solutions (1.54×10^{-5} M in acetone, 400 μ l) are added to containers, and the acetone is allowed to evaporate. Ten mL aqueous polymer solutions at different concentrations are then added to the containers containing pyrene residue. All the aqueous sample solutions contain excess pyrene residue at the same concentration of 6.17×10^{-7} M. The solutions are kept for 24 h at 20°C to reach the solubilization equilibrium of pyrene in the aqueous phase. Excitation is carried out at 340 nm, the emission spectra are recorded from 350 to 500 nm. Both excitation and emission bandwidths are 8 nm. From the pyrene emission spectra, the intensity (peak height) ratios (I_3/I_1) of the third band (391 nm, I_3) to the first band (374 nm, I_1) are analyzed as a function of polymer concentration. A CMC value is taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations.

Size of micelles

Dynamic light-scattering (DLS) measurements are carried out using a N4 Plus instrument (Coulter) equipped with a He-Ne laser (632 nm) to determine size of micelles.

Transmission electron microscope (TEM) measurements

A drop of resultant micelle solutions containing 0.01% phosphotungstic acid is placed on a copper grid coated with a polymer film, and self dried at 20°C. The TEM observations are carried out on a Philips CM300 or a JEM-2010 microscope with an electron kinetic energy of 200 kV and 300 kV, respectively.

Encapsulation of cyclosporin, a model hydrophobic drug, into the polymeric micelles and determination of cyclosporin encapsulation efficiency

A 15 mg of polymer 4 is dissolved in 5 mL DMF and 2-6 mg cyclosporin (CyA) is added to the polymer solution. The mixture is placed in a dialysis bag with Mw cut off of 2000 and immersed in 500 mL water at various temperatures (5, 10, 15 and 20°C). Water is replaced every hour up to 8 hours and the dialyzed sample is allowed to equilibrate up to 24 hours. The micelles formed are freeze-dried and stored at 4°C.

The resultant CyA-loaded micelles are dissolved in 1 mL acetonitrile and shaken for 1 hour. The sample is then centrifuged at 10,000 rpm for 10 min. The supernatant is filtered with 0.2 μm filter and analyzed by a high performance liquid chromatography method (HPLC). The HPLC system consists of a Waters 2690 separation module and a
 5 996 PDA detector. Waters SymmetryShield TM C8 column (15 x 3.9 cm) fitted with guard column is used as the separation column. The column and sample temperatures are set at 50 and 15°C respectively. The mobile phase consists of acetonitrile and water with volume ratio of 85 to 15. The flow rate of the mobile phase is fixed at 1 mL/min. The maximum injection volume is 100 μL and the UV detection is at 210 nm.
 10 The encapsulation efficiency is calculated as the weight ratio of actual to theoretical CyA content.

In vitro release tests of CyA-loaded micelles

A certain amount of CyA-loaded micelles is immersed in 50 mL PBS (pH7.4) at 10 and
 15 40°C (under sink condition). At fixed time intervals, the in vitro medium is replaced with fresh PBS. The collected specimen is extracted with 5 mL DCM. The extraction efficiency is higher than 95%. The organic phase is dried to harvest CyA, which is then dissolved in 2 mL of acetonitrile and water having a volume ratio of 85 to 15 for further HPLC analysis.

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1.3 Results

Polymer Synthesis and Characterization

The precursor polymers are obtained in 80.8 - 82.6% yield. The molecular weights of
 25 the hydroxyl-terminated precursor polymers are controlled by the feed ratio of the chain-transfer agent relative to the two monomers. Two products with molecular weights of 3,060 and 7,970 (Mw) and polydispersities of 1.2 and 1.5 are synthesized, respectively. The glass transition temperatures (T_g) of the high and low Mw polymers are determined to be 128.8 and 127.7°C, respectively. The aqueous solutions of the two polymers
 30 exhibit phase-transition at temperatures of 39.5 and 40.6°C, respectively. The ^1H NMR spectra of the two-hydroxyl-terminated polymers share a similar pattern. For clarity, only the ^1H NMR spectra of the low Mw polymer (1) in CDCl_3 is shown in Figure 1. The success of the polymerization is evidenced by the absence of vinylic proton signals at δ 5.4-6.6. Other signals from the iso-propyl groups (CHMe_2 at δ 4.05 and CHMe_2 at δ
 35 1.15, Signals e and a, respectively) and NMe_2 groups at δ 2.92 (Signal d) are observed and their chemical shifts are similar to those of the monomers. In addition, broad peaks

at δ 1.5-2.8 (Signal b+c) are observed for the prochiral methylene protons (CHCH_2) of both polymerized monomers and the methine protons ($\text{CHCH}_2\text{CONHCHMe}_2$) of NIPAAm units, as a consequence of the polymerization of C=C double bonds. Based on the integration of NMe_2 (Signal d) and $\text{CH}_2\text{CHCONMe}_2$ (Signal c') of DMAAm units, and the fact that the integration of CHCONHCHMe_2 (Signal c) and $\text{CH}_2\text{CHCOCHMe}_2$ (Signal e) is 1:1, the weak peak at δ 3.10 is assigned to $\text{CH}_2\text{CHCONMe}_2$ (Signal c') of DMAAm units. From the integration of the signals c' + d to the signal e, the m/n value of the polymer is estimated to be 7:1, which is approximately equal to the feed ratio of the two monomers. This indicates that the two monomers exhibit similar reactivity in the polymerization reactions. The IR spectra of the two hydroxyl-terminated polymers are also similar. For instance, the IR spectrum of polymer 1 exhibits strong absorptions at 3502.9cm^{-1} ($\nu_{\text{N-H}} + \nu_{\text{O-H}}$), 1654.8 cm^{-1} ($\nu_{\text{C=O}}$) and 1546.2 cm^{-1} ($\nu_{\text{C-N}}$). Subsequently, the hydrophobic cholesteryl group is conjugated to the precursor polymers. The two blocks are linked by a potentially hydrolytically labile carbonate bond, rendering the designed polymers biodegradable in aqueous media. Thus two cholesteryl-terminated polyacrylamide derivatives with different hydrophobic/hydrophilic lengths ratio are synthesized. The cholesteryl-terminated polymers (3 and 4) are purified by ultrafiltration, and are obtained as fine white plates in 70.2 and 72.9% yields, respectively. The Mw values of 3 and 4 are 3370 and 8030 with the polydispersity values being 1.2 and 1.3, respectively. The Tg values of 3 and 4 are 133.1 and 136.0°C, respectively. The glass transition temperatures of the cholesteryl-terminated polymers are significantly higher than those of the hydroxyl-terminated precursor polymers, possibly due to the additional hydrophobic-hydrophobic interactions between cholesteryl moieties. The successful conjugations of cholesteryl groups with the hydroxyl-terminated precursors are also confirmed by spectroscopic studies. For example, in the ^1H NMR spectra of the diblock polymers (Figure 2), signals from the five CH_3 groups (Signals g, h, i, and j) and the vinylic proton in the cholesteryl unit (Signal k) are observed. These signals share similar chemical shifts and coupling patterns as their corresponding signals of cholesteryl chloroformate. The signals from the Me group at the α position of C=C bond and the vinylic proton in the cholesteryl unit of the diblock polymers shift slightly to a higher field relative to cholesteryl chloroformate. The IR spectra of the two cholesteryl-terminated products share similar patterns as the spectra of the precursor polymers. However, the absorptions of $\nu_{\text{N-H/O-H}}$ (3436.6 cm^{-1}) and $\nu_{\text{C=O}}$ (1648.6 cm^{-1}) in the cholesteryl-terminated products red-shift slightly. Furthermore, the intensity of the peak at 3436.6 cm^{-1} decreases slightly after conjugation, indicating the

consumption of the hydroxyl group in the precursor polymers. The two cholesteryl-terminated polymers are highly soluble in both water and dichloromethane.

Behaviours in aqueous solution

- 5 The LCST values of the low Mw and high Mw polymer solutions were 37.7 and 38.2°C, respectively. The LCST can be adjusted by varying the ratio of NIPAAm to DMAAm. Since N, N-dimethylacrylamide is more hydrophilic, an increase in its content leads to a higher LCST. The CMC values for the low Mw and high Mw polymers are determined to be 9 and 25 mg/L, respectively. This indicates that the polymer with a higher
10 hydrophilic/hydrophobic length ratio exhibits a higher CMC value.

Table 1 summarizes the properties of the two hydroxyl-terminated poly(N-isopropylacrylamide-co-N, N-dimethylacrylamide) precursor polymers and the resultant cholesteryl-terminated block copolymers as well as their behaviours in aqueous solution.

15

Table 1. Synthesis and characterization of the precursor and cholesteryl-terminated polymers

Polymers	[NIPAAm] /[DMAAm] /[CTA] ^a	Mw	Mn	Mw/Mn	Tg (°C)	LCST (°C)	CMC (mg/L)
1	7:1:0.15	3060	2620	1.2	127.7	40.6	-
2	7:1:0.08	7970	5500	1.5	128.8	39.5	-
3	-	3370	2930	1.2	133.1	37.8	9
4	-	8030	6420	1.3	136.0	38.2	25

a. Molar ratio of the monomers and the chain transfer agent 2-hydroethanethiol.

Micelle formation

- 20 Micelles are fabricated using both the diblock polymers (3 and 4) by the membrane dialysis method. An initial concentration of 0.35% in weight in DMF is used for both polymers. The TEM images of micelles prepared from low and high Mw diblock polymers are shown in Figure 3. Most of the micelles are observed as cuboid-like shapes, though a few spherical particles are also observed. The size (taken as the
25 average of the length and breadth of individual micelle) is in the ranges of 45-80 nm and 70-110 nm for the low Mw and high Mw polymers, respectively. Under the same conditions, micelles self-assembled from the high Mw polymer are significantly larger than those prepared from the low Mw polymer. In addition to 0.35%, initial concentrations of 0.1% and 1.2% of polymer 3 in DMF are also tested. The TEM
30 pictures of the micellar aggregates formed at these polymer concentrations are

presented in Figure 4. Unlike the micellar aggregates formed from 0.35% initial solution, the micelles formed at an initial concentration of 0.1% are spherical with smaller size being in the range of 30-50 nm (Fig. 4a), whereas the micelles formed at 1.2% exhibit multiple morphologies (Fig. 4b, 4c and 4d). The 1.2% initial solution yields a large number of star-like micelles ranging 65-140 nm in size. A few large cuboid-like particles with sizes greater than 245 nm are also observed. The star-like nanoparticles are characterized by a core and needles pointing outwards. The observation under a higher magnification shows that the cuboid-like particles have a dense core and a relatively loose periphery (Fig. 4d)

Encapsulation of cyclosporin, a model hydrophobic drug, into the polymeric micelles

Figure 5 shows the SEM pictures of CyA-loaded micelles made from the polymer 4 at different temperatures. A high dialysis temperature (e.g. 20°C) yields cuboid-like micelles but a low dialysis temperature produces spherical micelles. Table 2 lists the size and encapsulation efficiency of CyA-loaded micelles. A decreased dialysis temperature results in larger size and higher encapsulation efficiency of CyA-loaded micelles. The lower the initial CyA loading, the greater the encapsulation efficiency of CyA. 64% encapsulation efficiency of CyA is yielded when the dialysis temperature of 10°C and the initial CyA loading of 3 mg are employed.

Table 2. Mean diameter and encapsulation efficiency (EE) of the CyA-loaded micelles made from the polymer 4 at different dialysis temperatures

Dialysis temp. (°C)	CyA Fed (mg)	Loading level (w/w%)	EE (%)	Mean diameter (nm)
20	6	1.9	5	41
15	6	6.5	17	173
15	3	6.5	32	143
10	6	12	32	169
10	3	11	64	--
5	6	15.6	36	193

In vitro release of CyA-loaded micelles

The in vitro release tests of CyA-loaded micelles are performed at two different temperatures. Figure 6 shows the release profiles of CyA from the micelles. At 40°C, which is above the LCST, CyA release is much faster compared to 10°C. At the temperature of higher than the LCST, the P(NIPAAm-co-DMAAm) block becomes hydrophobic. Thus the micelles de-associate and trigger off CyA release.

EXAMPLE 2: Cholesteryl-grafted poly(*N*-isopropylacrylamide-co-*N*-(hydroxymethyl)acrylamide) (*P*(NIPAAm-co-NHMAAm)) temperature-sensitive micelles

2.1 Materials

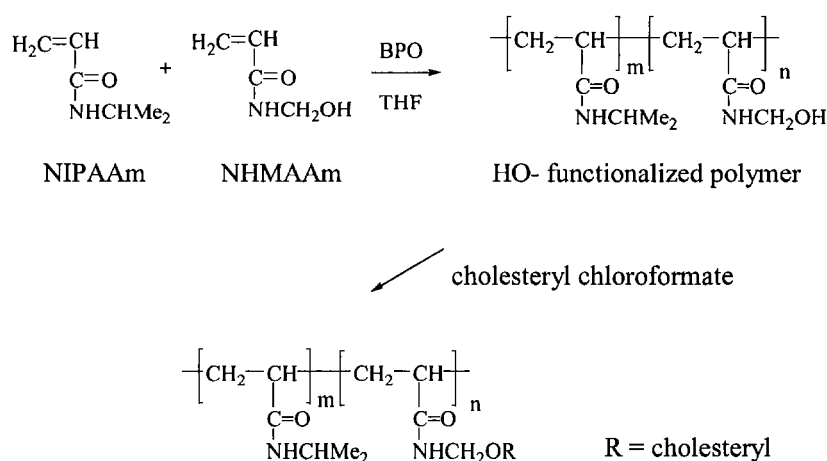
N-(hydroxymethyl)acrylamide is obtained from Aldrich. The rest chemicals are the same as those used for cholesteryl-terminated *P*(NIPAAm-co-DMAAm) micelles.

2.2 Methods

Synthesis of amphiphilic polymers of cholesteryl-grated (*P*(NIPAAm-co-NHMAAm))

The cholesteryl-grafted *P*(NIPAAm-co-NHMAAm) is produced by two steps (Scheme 3). First, *P*(NIPAAm-co-NHMAAm) is synthesized by the co-polymerization of *N*-isopropylacrylamide and *N*-(hydroxymethyl)acrylamide using BPO as the initiator. The NHMAAm is used as the functional unit to provide hydroxy groups. Subsequently, the cholesteryl units are conjugated with the hydroxy groups in the precursor polymer by the facile esterification reaction.

Scheme 3



Characterization methods are the same as those employed for the cholesteryl-terminated *P*(NIPAAm-co-DMAAm) polymers.

2.3 Results

Successful graft of cholesteryl group to the precursor polymer is proven by GPC studies (Figure 7) and ¹H NMR studies (Figure 8). From the NMR spectrum, each polymer molecule contains about 3-4 cholesteryl groups. The average molecular mass of the

cholesteryl-grafted P(NIPAAm-co-NHMAAm) is about 10.6kDa. Its polydispersity index is around 1.56, and its LCST in water is determined to be 40.8°C.

The CMC value of the cholesteryl-grafted polymer is determined to be 4mg/L, which is much lower than that of the cholesteryl-terminated polymer, indicating that the micelles made from the cholesteryl-grafted polymer are more stable.

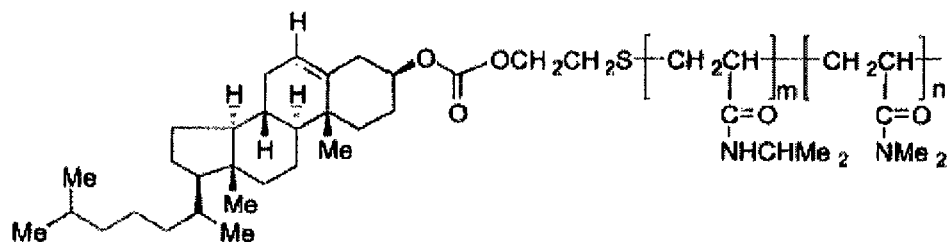
5 Cyclosporin is encapsulated within the cholesteryl-grafted P(NIPAAm-coNHMAAm) micelles. Figure 9 shows the TEM pictures of the blank and CyA-loaded micelles made from cholesteryl-grafted P(NIPAAm-co-NHMAAm), which are dialyzed at 10°C. Both the blank and CyA-loaded micelles exhibit a cuboid-like shape, and have a size ranging
10 from 100 to 200 nm.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described herein without departing from the spirit or scope of the invention. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A polymeric micelle system, for encapsulating a hydrophobic substance, wherein
5 each polymeric micelle is formed of block copolymers each having a temperature-sensitive block and a cholesteryl moiety, wherein the cholesteryl moieties form a hydrophobic core of the micelle and the temperature-sensitive blocks form a hydrophilic shell of the micelle.
2. A polymeric micelle system according to claim 1, wherein the temperature-sensitive block consists of a temperature-sensitive segment and a more hydrophilic
10 segment.
3. A polymeric micelle system according to claim 2, wherein the temperature-sensitive segment is poly(N-isopropylacrylamide) ("PNIPAAm") or one of its derivatives.
4. A polymeric micelle system according to claim 3, wherein the derivative of
15 PNIPAAm is poly(N,N-diethylacrylamide).
5. A polymeric micelle system according to any preceding claim, wherein the more hydrophilic segment comprises one or more functional groups for ligand conjugations.
6. A micelle system according to any preceding claim, wherein the more hydrophilic segment is selected from the group of molecules consisting of poly(N,N-
20 dimethylacrylamide) ("PDMAAm"), poly(N-(hydroxymethyl)acrylamide) ("PNHMAAm"), poly(acrylic acid), poly(vinylacetic acid) and their derivatives.
7. A polymeric micelle system according to claim 6, wherein the more hydrophilic segment is PDMAAm.
8. A polymeric micelle system according to claim 6, wherein the more hydrophilic
25 segment is PNHMAAm.
9. A micelle system according to claim 6, wherein the derivatives of the molecules include those containing one or more amine groups, hydroxyl groups or carboxylic acid groups.
10. A micelle system according to claim 9, wherein the derivative of the molecule is
30 poly((dimethylamino)ethyl methacrylate).
11. A polymeric micelle system according to any preceding claim wherein the cholesteryl moiety is cholesterol.
12. A polymeric micelle system according to any preceding claim wherein the cholesteryl moiety and the temperature-sensitive block are linked by a carbonate bond.

13. A polymeric micelle system according to claim 7 wherein the block copolymer is cholesteryl-terminated poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) having the formula:



5 wherein the ratio of m to n is in the range of 100:1 to 1:100.

14. A polymeric micelle system according to claim 13 wherein the ratio of m to n is about 7:1.

15. A polymeric micelle system according to any preceding claim wherein the micelles are capable of undergoing a phase transition at a lower critical solution
10 temperature ("LCST") in the range of from about 32°C to about 50°C.

16. A polymeric micelle system according to claim 15, wherein the LCST of the micelles is in the range of from about 37°C to about 42°C.

17. A polymeric micelle system according to any preceding claim wherein said system has a critical micelle concentration in the range of from 4 mg/L to 200 mg/L.

15 18. A polymeric micelle system according to any preceding claim, further comprising a hydrophobic substance encapsulated with the polymeric micelles.

19. A polymeric micelle system according to claim 18 wherein the hydrophobic substance is a drug selected from the group consisting anti-cancer drugs, antiphlogistic anodynes, immuno-suppressants, hepatism remedies, hormone compositions, chemo-
20 therapeutics, metabolic pharmaceuticals, digestive disease remedies, respiratory disease remedies, anti-allergic pharmaceuticals, central nervous system disease remedies, peripheral disease remedies, circulatory disease remedies and anti-fungal remedies.

20. A polymeric micelle system according to claim 19 wherein the anti-cancer drugs
25 are selected from the group consisting of paclitaxel, doxorubicin, teniposide, etoposide, daunomycin, methotrexate, mitomycin C, indomethacin, ibuprofen, cyclosporin, biphenyl

dimethyl dicarboxylate, triamcinolone acetonide, betamethasone, amphotericin B, ketoconazole, itraconazole, daunorubicin, griseofulvin, foroprimidine, lidocaine, epirubicin, vincristine, vinblastine, ellipticine, camptothecin, docetaxol, cisplatin, prednisone, and methyl-prednisone.

5 21. A polymeric micelle system according to claim 20 wherein the drug is paclitaxel, doxorubicin or cyclosporin A.

22. A method of preparing the block copolymers of any one of claims 1 to 7, comprising the following steps:

10 (a) synthesising hydroxyl-terminated poly(N-isopropylacrylamide-co-N, N-dimethylacrylimide) precursor polymers; and

(b) reacting the precursor polymers with cholesteryl chloroformate.

23. A method according to claim 22, wherein the reaction is effected by dissolving the cholesteryl chloroformate, the precursor polymers and a catalyst in a solvent to form a reaction mixture.

15 24. A method according to claim 23, wherein the solvent is dichloromethane or tetrahydrofuran.

25. A method according to claim 23 or claim 24, wherein the reaction mixture is stirred for about 24 hours at room temperature.

20 26. A method according to claim 24 or claim 25 wherein about 1 gram of hydroxyl-terminated P(NIPAAm-co-DMAAm), about 0.5 grams of cholesteryl chloroformate and about 0.1 gram of triethylamine are dissolved in 30 mls CaH₂ – dried dichloromethane.

25 27. The method of any one of claims 22 to 26 wherein, before step (a), the precursor polymers are synthesised by radical copolymerisation of N-isopropylacrylamide and N,N-dimethylacrylamide monomers, in the presence of an initiator and a chain transfer agent.

28. A method according to claim 27, wherein the initiator is benzoyl peroxide.

29. A method according to claim 27 or claim 28, wherein the chain transfer agent is 2-hydroxyethanethiol.

30 30. A method of preparing the block copolymers of any one of claims 1 to 6 and 8 comprising the following steps:

(a) synthesising poly(N-isopropylacrylamide-co-N-(hydroxymethyl)acrylamide precursor polymers; and

(b) reacting the precursor polymers with cholesteryl chloroformate.

35 31. A method according to claim 30, wherein the reaction is effected by dissolving the cholesteryl chloroformate, the precursor polymers and a catalyst in a solvent to form a reaction mixture.

32. A method according to claim 31, wherein the solvent is dichloromethane or tetrahydrofuran.
33. A method according to claim 31 or claim 32, wherein the reaction mixture is refluxed at about 70°C for about 6 hours.
- 5 34. A method according to any one of claims 30 to 33, wherein, before step (a), the precursor polymers are synthesised by co-polymerising NIPAAm and NHMAAm in the presence of an initiator.
35. A method according to claim 34 wherein the initiator is benzoyl peroxide.
36. A method according to any one of claims 22 to 35 wherein the reaction of step
10 (b) is an esterification reaction.
37. A method according to claim 36 wherein the esterification reaction is carried out at room temperature.
38. A method according to any one of claims 23 to 29 or any one of claims 31 to 37, wherein the catalyst is triethylamine.
- 15 39. A method according to any one of claims 22 to 38, further including a precipitation step to precipitate the block copolymers from the reaction mixture.
40. A method according to claim 39, wherein precipitation is effected by adding diethyl ether to the reaction mixture.
41. A method according to claim 39 or claim 40 further including a purification step
20 wherein the precipitated products are purified by ultra-filtration using a filtration membrane.
42. A method according to claim 41, wherein the filtration membrane has a molecular weight cut-off of about 2000.
43. A method of preparing the polymeric micelles of the system according to any
25 one of claims 1 to 21, comprising the following steps:
- (a) dissolving the block copolymers in dimethylformamide ("DMF") to form polymer solutions; and
 - (b) dialyzing the polymer solutions against de-ionized water
44. A method according to claim 43 wherein the dialysis step is effected by
30 membrane dialysis.
45. A method according to claim 44, wherein the dialysis membrane has a molecular weight cut-off of about 2000.
46. A method according to any one of claims 43 to 45, wherein the initial concentration of the block copolymers in DMF is about 0.35% by weight.

47. A method according to claim 46 wherein the micelles formed are cuboid-like in shape and have a size in the range of about 45-80 nm for low Mw polymers and about 70-110 nm for high Mw polymers.
48. A method according to any one of claims 43 to 45, wherein the initial concentration of the block copolymers in DMF is about 0.1% by weight.
49. A method according to claim 48 wherein the micelles formed are substantially spherical having a size in the range of about 30-50 nm for low Mw polymers.
50. A method according to any one of claims 43 to 45, wherein the initial concentration of the block copolymers in DMF is about 1.2% by weight.
51. A method according to claim 50 wherein the micelles formed are substantially star-shaped and have a size in the range of about 65-245 nm for low Mw polymers.
52. A method for encapsulating a hydrophobic substance into polymeric micelles of a micelle system according to any one of claims 1 to 21 comprising the following steps:
- (a) preparing a polymer solution of the block copolymers in DMF;
 - (b) adding a quantity of the hydrophobic substance to the polymer solution to form a loaded polymer solution; and
 - (c) dialyzing the loaded polymer solution against de-ionised water.
53. A method according to claim 52, wherein the dialysis step is effected by membrane dialysis using a dialysis membrane.
54. A method according to claim 53, wherein the dialysis membrane has a molecular weight cut-off of about 2000.
55. A method according to claim 54, wherein the dialysis membrane is a dialysis bag and the loaded polymer solution is placed in the dialysis bag.
56. A method according to claim 55, wherein the dialysis bag is immersed in about 500 mls of de-ionised water.
57. A method according to claim 56, wherein the dialysis bag is immersed in the water for up to 8 hours, with the water being replaced about every hour.
58. A method according to any one of claims 52 to 57, wherein the dialysis step is carried out at a temperature in the range of about 5°C to a temperature just below the LCST.
59. A method according to claim 58 wherein the dialysis temperature is about 10°C.
60. A method according to any one of claims 52 to 59, wherein the hydrophobic substance is a drug selected from the group consisting of anti-cancer drugs, antiphlogistic anodynes, immuno-suppressants, hepatitis remedies, hormone compositions, chemo-therapeutics, metabolic pharmaceuticals, digestive disease remedies, respiratory disease remedies, anti-allergic pharmaceuticals, central nervous

system disease remedies, peripheral disease remedies, circulatory disease remedies and anti-fungal remedies.

61. A method according to claim 60, wherein the anti-cancer drugs are selected from the group consisting of paclitaxel, doxorubicin, teniposide, etoposide, daunomycin,
5 methotrexate, mitomycin C, indomethacin, ibuprofen, cyclosporin, biphenyl dimethyl dicarboxylate, triamcinolone acetonide, betamethasone, amphotericin B, ketoconazole, itraconazole, daunorubicin, griseofulvin, forpyrimidine, lidocaine, epirubicin, vincristine, vinblastine, ellipticine, camptothecin, docetaxol, cisplatin, prednisone, and methyl-prednisone.
- 10 62. A method according to claim 61, wherein the drug is paclitaxel, doxorubicin or cyclosporin A.
63. A method according to claim 62 wherein the drug is cyclosporin A and the encapsulation efficiency of cyclosporin A within the micelle system is up to about 64%.
64. A method of synthesizing block copolymers having a desired LCST, for use in
15 the micelle systems of any one of claims 1 to 7, comprising the following steps:
 - (a) determining a copolymer ratio of the temperature-sensitive segment to the more hydrophilic segment, in the block copolymer, which is required so that the block copolymer exhibits an LCST which is about the desired LSCT;
 - 20 (b) preparing a reaction solution comprising monomers corresponding to the temperature-sensitive segment, monomers corresponding to the more hydrophilic segment, an initiator and a chain transfer agent, wherein the molar ratio of the two types of monomers is substantially equal to the copolymer ratio; and
 - 25 (c) heating and mixing the reaction solution.
65. A method of synthesizing block copolymers having a desired LCST, for use in the micelle systems of any one of claims 1 to 6 and 8, comprising the steps of claim 64, except that the reaction solution of step (b) does not include a chain transfer agent.
66. A method according to claim 64 or claim 65, wherein the temperature-sensitive
30 segment is PNIPAAm or one of its derivatives.
67. A method according to any one of claims 64 to 66, wherein the more hydrophilic segment is selected from the group of molecules consisting of poly(N, N-dimethylacrylamide) ("PDMAAm"), poly(N-hydroxymethyl) acrylamide ("PNHMAAm"), poly(acrylic acid), poly(vinylacetic acid), and their derivatives.
- 35 68. A method according to claim 67, wherein the more hydrophilic segment is PDMAAm.

69. A method according to claim 67 wherein the more hydrophilic segment is PNHMAAm.
70. A method according to claim 67, wherein the derivatives of the molecules include those containing one or more amine groups, hydroxyl groups or carboxylic acid groups.
71. A method according to claim 70, wherein the derivative of the molecule is poly((dimethylamino)ethyl methacrylate).
72. A method according to any one of claims 64 to 71, wherein the more hydrophilic segment comprises one or more functional groups for ligand conjugations
73. A pharmaceutical composition comprising a polymeric micelle system according to any one of claims 1 to 21, and a pharmaceutically acceptable carrier, wherein said micelle system is loaded with a therapeutically effective amount of a hydrophobic drug.
74. A composition according to claim 73, for curing, preventing, treating or alleviating cancer, wherein the hydrophobic drug is an anti-cancer drug.
75. A composition according to claim 74, wherein the anti-cancer drug is paclitaxel, doxorubicin or cyclosporin A.
76. A method of treating a patient to cure, prevent, treat or alleviate a disease, said method comprising administering to said patient a composition according to any one of claims 73 to 75.
77. A method according to claim 76, wherein the disease is cancer and the hydrophobic drug is an anti-cancer drug.
78. A method according to claim 77, wherein the anti-cancer drug is paclitaxel, doxorubicin or cyclosporin A.
79. A method according to any one of claims 76 to 78, wherein the composition is administered orally, topically, by injection or by suppository.
80. A method according to any one of claims 76 to 79, further including the step of heating targeted tissue or a targeted organ to a temperature at or above the LCST so as to cause the hydrophobic drug to be released from the micelles.